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A feeding trial with large salmon

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NUTRITION AND THE HITRA DISEASE
THE BJUGN PROJECT
A feeding trial with large salmon

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The feeding trial was begun in Bjugn in September 1984 and concluded in May 1985, while the analytical work was finished at the end of 1985.

During the trial period, Einar Lied was on a leave of absence, working as a visiting scientist in Sweden and Scotland. Lie and Sandnes coordinated and directed the execution of the Project in cooperation with the Project Group. Sampling in Bjugn was carried out by Waagbo, Sorheim, Sandnes, Lie, and Magny Karlsen, D. Eng. .

The analytical work was mainly carried out by Waagbo (clinical and hematological analyses) and Sorheim (raw analyses), both of whom were assigned to the Project with Bjugn Industrier A/S as their employer. Further, analyses were conducted with amino acids (Njaa), vitamin E and fatty acids (Lambertsen), minerals (Julshamn) and vitamin C (Sandnes), while Utne was responsible for feeds and feed composition.

The results were processed by Waagbo in cooperation with Lie and Sandnes.

The present report was written mainly by Lie, Sandnes and Waagbo, with contributions by the other members of the Project Group as indicated by their areas of responsibility.

The report was edited by Njaa and Lambertsen.

Through the Bjugn Project, the Nutrition Institute has been connected with the Healthy Fish (Frisk Fisk) Group, to which it has reported. Some parts of the results have been published in four articles in The Norwegian Fish Breeding (Norsk Fiskeoppdrett) in 1985 - 1986.

The Nutrition Institute wishes to thank Lars Aglen, previously manager at Bjugn Industrier A/S, for his contribution towards making this Project possible. Aglen and the employees of Bjugn Industrier A/S deserve special thanks for their enthusiasm and inputs during the execution of the Project.

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F. Hoffman - La-Roche : G.O. Johnsen, South Trondelag County.

We dedicate this joint venture of our Institute to chief scientific officer
Olaf R. Braekkan, Ph. D., who retired on 30.06.1986.

Leif Rein Njaa
Acting chief scientific
officer

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I V SUMMARY

1. The Bjugn Project aimed to make clear the significance of nutrition in outbreaks of Hitra disease. A feeding trial with large salmon (aver. weight 1.4 kg) was started in 10 enclosures in September 1984 to be concluded in May 1985. The fish were sampled eight times at even intervals in the course of the trial period. Each sample consisted of 20 fishes per group for analysis and another 10 fishes to gather more data on weight and length. The sampling material was complemented by samples of healthy and Hitra-diseased salmon from a commercial fish farm.

2. The following feed types and additives were used :

Group 0 : Control feed based on soft pellets of regular silage and pellet meal with 44 % metabolizable energy from protein and 35 % from fat.

Group 1 : Feed based on evaporated silage from Bjugn Industrier A/S.

Group 2 : Tess Edelfor feed (Skretting).

Group 3 : Feed composed of evaporated silage, protein concentrate, and pellet meal. Metabolizable energy from protein 52 % and from fat 25 %.

Group 4 : Evaporated silage, capelin oil and pellet meal with 36 % of energy from protein and 48 % from fat.

Group 5 : Control feed (Group 0) with a 50 % admixture of methionine (amino acid).

Group 6 : Control feed (Group 0) with 30 % admixture of vitamin E and 4-fold increase of selenium content.

Group 7 : Control feed (Group 0) with 30 % admixture of vitamin E.

Group 8 : Control feed (Group 0) with 4-fold increase of selenium content.

Group 9 : Control feed (Group 0) with admixture of 3 000 mg vitamin C per kg feed.

3. Samples were subjected to the following analyses :

Blood tests. Hematology : No. of RBC, Hb, Hct, and Lct. Serum : AP, ASAT, ALAT, total protein, albumin, creatinine, triglycerides, cholesterol, iron, copper, zinc, selenium, vitamin E, amino acids and glutathione peroxidase.

Liver. Dry matter, protein, fat, ash, iron, copper, zinc, selenium, vitamin E, vitamin C and fatty acids.

Whole fish. Dry matter, protein, fat and ash.

Fillet. Dry matter, protein, fat, ash, amino acids and fatty acids.

Feed. Dry matter, protein, fat, ash, iron, copper, zinc, selenium, vitamin E, vitamin C, methionine and fatty acids.

Also, round fish, liver, milt, and in some cases heart, were weighed. Liver and muscle samples were studied histologically at the Veterinary Institute. A selection of serum samples were analyzed for specific bacterial protein VS-Pl at the Research Institute of the University of Tromsø (=FORUT).

4. Normal, healthy farmed salmon with less than one year's growing period in the sea can be characterized by the following blood values :

Hematology : RBC $0.85 - 1.15 \times 10^{12}/L$; Hb $9.0 - 11.0 \text{ g}/100 \text{ mL}$; Hct $44 - 50 \%$; Lct $0.3 - 1.0 \%$; MCV $400 - 550 \times 10^{-15} L$; MCH $95 - 110 \times 10^{-6} \text{ g}$; MCHC $20.0 - 22.0 \text{ g}/100 \text{ mL}$. Serum : total protein $40 - 55 \text{ g}/L$; albumin $18 - 24 \text{ g}/L$; albumin/protein $0.39 - 0.44$; creatinine $20 - 50 \text{ mmol}/L$; triglycerides $2.0 - 4.0 \text{ mmol}/L$; cholesterol $8.0 - 13.0 \text{ mmol}/L$; AP $600 - 1200 \text{ U}/L$; ASAT $200 - 400 \text{ U}/L$; ALAT $2 - 8 \text{ U}/L$. Other analyses of serum from control group (enclosure 0) showed the following values : selenium $0.17 - 0.25 \text{ mg}/L$; zinc $17 - 32 \text{ mg}/L$; copper $0.9 - 1.5 \text{ mg}/L$; iron $0.6 - 2.1 \text{ mg}/L$; and vitamin E $60 \text{ mg}/L$.

5. A feed with a high proportion of energy from fat (48 %) resulted in no negative effects on the health of the fish, while a feed with a low proportion of energy from fat (25 %) did not improve the salmon's health situation in this trial. The analytical parameters used for describing the physiological state were influenced neither by dry feed nor by feed composed of silage, evaporated silage and protein concentrate.

6. Salmon receiving a feed with more than 38 % metabolizable energy from fat did not deposit the excess fat in fillet but instead in intestines. Salmon fillet contains about 13 % fat (wet weight) with approx. 20 % polyunsaturated fatty acids (omega 3 series). These fatty acids are assumed to have a positive effect on human health.
7. Methionine, selenium, vitamin E, selenium/vitamin E and vitamin C added in quantities exceeding their normal proportions in feeds did not affect the hematological and clinical parameters used for describing health of fish. The additions resulted in elevated values of the added components in organs of the fish.
8. As the fishes showed no signs of Hitra disease in February 1985, 20 fishes from each enclosure were tagged, dip-treated with bacteria isolated from Hitra-diseased salmon, and returned to their enclosures. This work was carried out in cooperation with the Veterinary Institute which secured the bacterial culture. Blood samples were taken from the tagged fish at the time of the treatment, 1 week after it, and again 3 weeks after it.
9. No natural outbreaks of Hitra disease occurred in the facility during the trial period. The direct infection did not result in large-scale mortality either, even though the presence of salmon suffering of Hitra disease in the facility was verified by all cooperating research institutions. Relative mortality between the groups showed no correspondence with the health parameters previously mentioned.
10. Salmon with Hitra disease were characterized by sharply lowered values of the hematological parameters RBC, Hb and Hct, which means that the fishes were anemic.
11. Levels of the serum parameters total protein, albumin, creatinine, triglycerides, and cholesterol in salmon with Hitra disease showed a drop parallel with the hematological parameters mentioned in Point 10. Enzymatic activities for ASAT and ALAT varied depending on the course of the disease. AP followed the pattern of the hematologic analyses. The specific bacterial protein VS-Pl was verified in serum from fishes that were defined as having Hitra disease on the basis of their behavior, but it was not verified in samples taken at an earlier stage from the same fishes that according to the blood analyses showed early signs of developing the disease.

12. Salmon with Hitra disease had higher weight of liver than healthy fish. The liver of sick fish had a greater water content and a greater fat content in dry matter than that of healthy fish, but it could not be characterized as fatty liver. The total selenium content of liver was the same in sick and healthy fish; selenium quantity per kg protein in liver was not different either. Similar findings were made for copper, zinc, and iron.

13. Salmon with Hitra disease had enlarged milt. Also, concentration of iron per weight unit was double the normal value from the sick fish. This means that the total iron content in the milt of salmon with Hitra disease can be six times higher than the normal value.

14. Levels of the branched amino acids valine, isoleucine, and leucine in muscle extract were clearly elevated in sick fish, while the level of serine was strongly reduced.

15. A collective evaluation of the development of Hitra disease, based on analyses of sick fish from the feeding trial and from a commercial facility lays the groundwork for the following hypotheses :

- a. Disease is initiated.
- b. Membrane damage (indicated by enzymological findings) occurs.
- c. Hemolysis not verified. Possible damaged red blood cells are removed efficiently from circulation by milt, which increases in size and iron content.
- d. Tissue damage develops, with consequent leakage from vascular system (indicated by reduction of parameters for whole blood and serum. Beginning ascites.) .
- e. Fish attempts to compensate loss of red blood cells by new synthesis (indicated by MCV).
- f. Membrane damages increase in extent, involving several organs. Bacteria verified (indicated by branched amino acids in muscle extract and by enzymatic activity in serum . VS-P1 can be verified in serum.).
- g. Behavioral disturbances occur. Fish dies.

On the basis of the material collected, it is reasonable to point out two possible ways of initiation of Hitra disease in salmon :

I) Unknown factors cause membrane damage making the fish less resistant to bacterial infections caused a.o. by *Vibrio salmonicida*.

II) Fish is infected by *Vibrio salmonicida*, which results in membrane damage.

The latter alternative is considered to be more probable, even though bacteria were not verified at the earlier stages of disease development in this trial.

16. The Bjugn Project included also measurements of growth and of the use of the different feed types and additives.

The following results were obtained :

Group	Final weight kg	Feed use in kg	Feed factor dry matter	PER	PPV
0	4.5	7.5	2.5 (1.6)	1.5	0.25
1	3.8	6.3	2.7 (1.9)	1.3	0.29
2	4.6	5.7	1.8 (1.7)	1.5	0.42
3	3.3	4.4	2.4 (1.8)	1.2	0.39
4	4.2	5.4	1.9 (1.6)	1.6	0.43
5	4.1	5.9	2.3 (1.4)	1.6	0.35
6	3.9	6.1	2.5 (1.5)	1.5	0.44
7	4.0	6.0	2.5 (1.5)	1.6	0.42
8	3.6	5.3	2.5 (1.6)	1.5	0.36
9	3.9	5.9	2.5 (1.6)	1.5	0.31

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